Multinuclear MRS of metabolic dynamics in the brain

Rolf Gruetter

Laboratory for functional and metabolic imaging (LIFMET), Ecole Polytechnique Federale de Lausanne; Departments of Radiology, Universities of Lausanne and Geneva; Centre d'Imagerie BioMedicale (CIBM)

Introduction

In vivo spectroscopy, especially at high fields, provides the investigator with a unique tool. One strength of the method lies in the deterministic nature of the NMR signal: Not only is it in general known at what chemical shift to expect a signal for a given compound, the spectral features, which can be quite complex, can be faithfully reproduced using either model solution and/or simulations. Additionally, the lineshape is subject to certain constraints of similarity between the different metabolites. Finally, some of the concentrations expected should conform to what is reasonably expected from the underlying neurochemistry. These characteristics can serve to allow a faithful measurement of metabolites in vivo. On one hand, the concurrent quantitative measurement by ^{1}H NMR spectroscopy of many important neurochemicals constituting the neurochemical profile allows for non-invasive insight into the neurochemical consequences of many diseases in human subjects, thereby providing an important translational link. On the other hand localized NMR spectroscopy of other stable isotopes provides a window on *in vivo* brain metabolism, with a unique chemical specificity and diversity of potential measurements not possible by other methods. The metabolism of stable isotopes can be followed non-invasively using NMR, although administration of the "tracer" requires a high isotopic enrichment, for ^{13}C typically above 50% in the precursor pool. Because NMR spectroscopy can be used to detect label in different molecules and different chemical positions, it offers the attractive possibility to follow metabolism of the precursor, labeled at one or more specific positions. Applications are predominantly focused on nuclei where a stable isotope is present at low natural abundance, examples include ^{2}H, ^{15}N, ^{19}F and ^{13}C (76).

Of course, sensitivity for NMR is low when considering the concentrations that are detectable, and the relative sensitivity of ^{13}C NMR is even lower. Nonetheless, despite the sensitivity disadvantage, as shall be discussed here, NMR spectroscopy, especially at high field, can provide unique insights into brain metabolism. Many consider a low sensitivity and thus low spatial resolution a significant disadvantage. However, it is our belief that the spatial resolution that can be achieved combined with the high specificity of the method allows to uniquely address a significant number of important biomedical problems/questions.

Glucose concentration, and distribution in the brain: transport physiology

Glucose is the single most important substrate required for normal brain function, and the brain relies on a continuous import of glucose from the blood, which must occur across the blood-brain-barrier. Glucose transport rates into the brain are thus indicative of the maximal sustainable rate of glucose consumption, CMR_{glc}. Aside from lactate and glycogen (12), brain glucose is the only sizable kinetic pool upstream of acetyl-CoA and the size of the brain glucose pool and its physical distribution space is important for the derivation of absolute metabolic fluxes and quantitative positron emission tomography studies (9; 77). It has been shown that steady-state glucose transport kinetics can be derived from the relationship between brain and plasma glucose (9; 34; 77; 78).

Glucose concentrations have been measured using ^{1}H and ^{13}C NMR spectroscopy (34; 79-84). For a CH group with homonuclear J-coupling, the sensitivity advantage of ^{1}H compared to ^{13}C is expected to be only 2-3 fold. The proximity of the ^{1}H resonances of glucose to that of water makes the measurement of brain glucose using ^{1}H NMR spectroscopy difficult, especially for the H1 resonance (60). Therefore, in cases where metabolism is followed by ^{13}C NMR, it may be advantageous to measure brain glucose content as well.

When measuring brain glucose transport kinetics, the magnitude of the physical distribution volume, i.e. the volume in the brain into which glucose can diffuse, can affect the interpretation of the derived glucose transport kinetics (85-87). The physical distribution volume of glucose has been assumed to equal the brain's water phase based on radiotracer and slice diffusion experiments, suggesting that transport of glucose across the cell membranes is diffusion-limited (87), although some fraction of the aqueous space might be more accessible on a short time scale (88). Interestingly a study concluded that the physical distribution volume of glucose must be large and consistent with that of the brain's water phase from the delayed change in brain glucose following a step function in plasma glucose (89). In a recent study the diffusion behaviour of glucose was measured using diffusion-weighted ^{1}H NMR spectroscopy (84) and it was found that glucose had a distinctly detectable signal even at very high diffusion weighting (Figure 7), consistent with a predominantly restricted diffusion behaviour, reflecting the primarily intracellular origin of the glucose signal. However, the diffusion signal characteristic was different from compounds whose concentration is almost exclusively intracellular, but similar to that of lactate, whose extracellular concentration under normal circumstances is probably similar to whole brain lactate concentrations. From the diffusion behaviour of the glucose signal it was concluded that approximately 20% of the NMR signal of glucose originated from the extracellular compartment, which reinforced the notion of a high physical distribution volume for glucose (90).

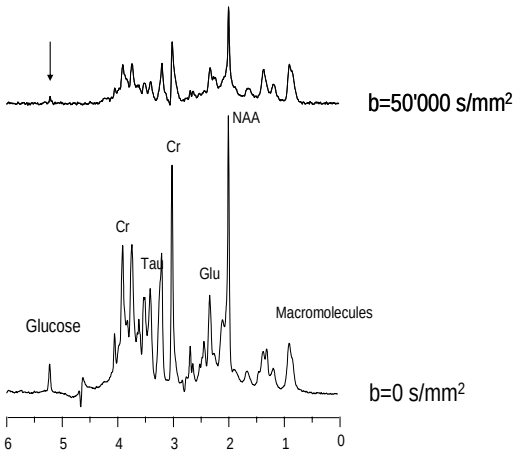

Figure 1 Diffusion weighted signal of glucose and other metabolites (84). Signals are strongly reduced at the high b value (diffusion time was 120 ms), more so than the macromolecule signal (at e.g. 0.9 ppm). The detection of the glucose signal at 5.23 ppm (arrow) indicates that a substantial fraction of the glucose is in intracellular space.

Traditionally, glucose transport kinetics have been analyzed with a model of brain glucose transport that was based on standard Michaelis-Menten kinetics. However, Michaelis-Menten kinetics is based on the assumption that initial rate of unidirectional product formation is measured, e.g., immediately after substrate and enzyme have been combined. This experimental condition would require the elimination of the brain glucose, which is difficult to achieve without interfering with normal brain function. Hence it is reasonable to expect that reversible Michaelis-Menten kinetics is a more appropriate formalism in describing brain glucose transport. Such a model has been proposed (34; 91). It was shown that one implication of the reversible model of brain glucose transport is that the relationship between brain and plasma glucose is linear (34). Many measurements of brain glucose content as a function of plasma glucose have in the meantime corroborated the observation that brain glucose concentrations are a linear function of plasma glucose (78; 81-83) and such a case is illustrated in Figure 8 for two different anesthetic regimes, α-chloralose and pentobarbital. These studies indicated that decreased electrical activity and thus decreased energy metabolism resulted in increased brain glucose concentrations. The increment in brain glucose was consistent with an approximately two-fold reduction in brain glucose utilization under pentobarbital anesthesia relative to α-chloralose anesthesia. The presence of a sizable concentration gradient between brain and plasma glucose implies that net glucose uptake (i.e. glucose consumption at steady-state) was appreciable even under conditions close to isoelectricity.

The importance of measuring the brain glucose concentration can be appreciated from its role in regulating brain glucose metabolism: Glucose becomes rate-limiting for metabolism when its concentration approaches that of the K_m of the first step in its metabolism, which is in the brain phosphorylation mostly by hexokinase. Since the K_m of brain hexokinase is very low (~50μM) and NMR sensitivity *in vivo* generally is too low to detect such small concentrations of glucose, brain glucose concentrations measured by NMR that are close to zero indicate metabolism that is limited by the glucose available to the brain cell. The general consensus is that brain glucose transport is not rate-limiting for metabolism under normal circumstances. We have recently shown in the conscious human and the α-chloralose anesthetized rat that the maximal sustainable rate of glucose consumption is approximately 60-90% above the basal rate of glucose metabolism and brain glucose is therefore not rate-limiting (34; 78). This may, however, not be the case under conditions of extreme metabolic activation or during hypoglycemia (92).

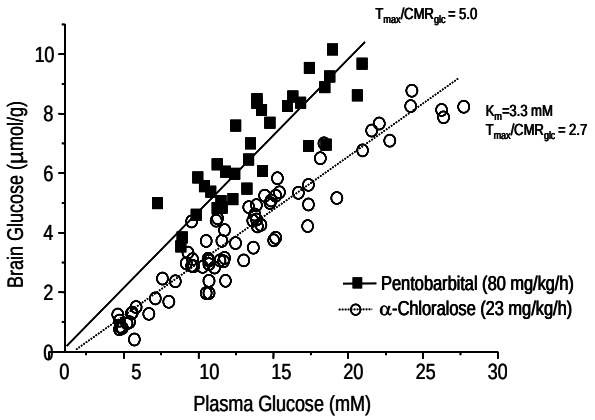

Figure 8 Brain glucose transport kinetics from the measurement of the brain glucose content as a function of plasma glucose concentration. (A) Demonstration of a linear relationship between brain and plasma glucose concentrations, as well as the effect of increased anesthesia (decreased electrical activity) on brain glucose content *in vivo*. From (81)

Previously, models of brain glucose transport have been evaluated at normal or hyperglycemic conditions only (34; 77; 81-83). A recent study extended the brain glucose concentration measurements to hypoglycemia using ^{13}C NMR spectroscopy (78). The concentrations measured by ^{13}C NMR were found to be in excellent agreement to those predicted by the reversible Michaelis-Menten model as well as those measured by ^{1}H NMR spectroscopy (78). Interestingly, when the brain glucose concentration approached zero, CBF was acutely increased (78) and glycogen degradation started (93), all of which points to brain glucose being important in activating cerebral defenses against a deficiency in fuel supply.

Brain glycogen, an endogenous energy store

Additional fuel can be provided to brain cells during e.g. hypoglycemia from glycogen, which is present in the brain in measurable quantities and appears to be essential for brain function. Brain glycogen is typically present in quantities that exceed those of tissue glucose in the brain. Similar to glucose, brain glycogen is rapidly eliminated in post-mortem tissue (12; 94; 95), therefore, its direct biochemical measurement is difficult (96; 97). Localized ^{13}C NMR spectroscopy has the unique capability of following brain glycogen metabolism longitudinally employing a much smaller number of animals than would be used with biochemical extraction.The rate of brain glycogen degradation during hypoglycemia accounted for the majority of the glucose supply deficit during the hypoglycemic period (93). Together with the apparent stability of glycogen in the non-stimulated brain at eu- or hyperglycemia (12; 98), these data suggest that brain glucose plays an important regulatory role in cerebral glycogenolysis. These studies also showed that brain glycogen increased above the basal level and beyond following a single episode of hypoglycemia (93). This rebound or super-compensation of brain glycogen may result in increased neuroprotection. It has been proposed that brain glycogen metabolism may be a factor involved in the mechanism of the hypoglycemia unawareness syndrome observed clinically in patients with type I diabetes (93; 98), perhaps through the enhanced neuroprotective effect of increased brain glycogen.

Thus glycogen likely is a viable and important store of glucose equivalents in the brain, whose metabolism is affected by hormones, neurotransmitters and second messengers (99).

One obvious investigational power of NMR spectroscopy is its applicability to animal models and humans alike. Because all previous studies measured brain glycogen metabolism in animals, the question

remained as to whether brain glycogen metabolism may be faster in the conscious human brain. Brain glycogen metabolism had never been measured in the human brain and ^{13}C NMR is the only technique that can provide this insight. We have recently adapted the localization method (see above on localization methods) for measuring brain glycogen in humans and demonstrated that a reproducible measurement of the brain glycogen signal was indeed possible in the human brain (14), illustrated in Fig. 9A. These initial results furthermore demonstrated that brain glycogen metabolism was extremely slow in subjects measured in the awake, resting condition (Figure 9B). This observation was in excellent agreement with previous studies showing that under the conditions of this study (plasma glucose at euglycemia or higher with concomitant hyperinsulinemia), the brain glucose concentration is well above the K_m of hexokinase (9; 34; 83), thereby eliminating the need for appreciable glycogen activation. In fact, the flux through glycogen synthase was estimated at 0.1-0.2 µmol/g/h. As a consequence, a brain glycogen pool of a few mM is expected to have a turnover time on the order of several days to a week. These findings suggest that glycogen metabolism is a negligible factor in the energy metabolism of the conscious unstimulated human brain at euglycemia and above.

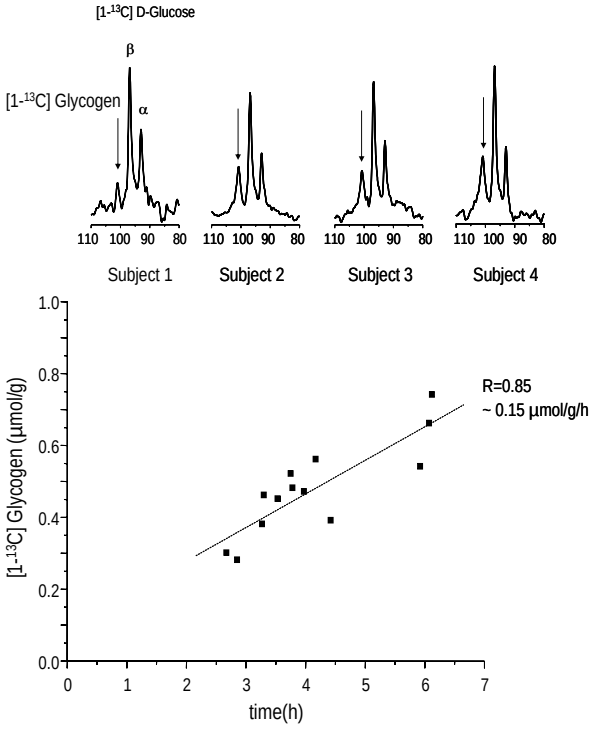

Figure 9 Measurement of glycogen in the human brain. (A) Demonstrates the detection of the brain glycogen signal in four different subjects (arrows) along with the glucose C1 resonances. Shown is the spectral region containing the glycogen C1 and glucose C1 resonances. (B) The increase in the quantified glycogen C1 signal represents the accumulation of [1-^{13}C] glycogen, which occurred at an extremely slow rate on the order of 0.15µmol/g/h in the human brain, as illustrated in the graph containing measurements from three different studies. From (14).

Compartmentalized oxygen metabolism in brain

The question whether there is tight coupling between glucose and oxygen consumption in the brain has become of paramount importance. The landmark study by Fox and Raichle in the late 1980's suggested that there is indeed a large increase in glucose metabolism that exceeds the changes in oxygen metabolism (100). The concept of uncoupled oxygen metabolism has been supported by studies reporting small increases in brain lactate during focal activation (101; 102) that are very difficult to perform (103). The relatively small magnitude of change in brain lactate (101; 102; 104) is difficult to reconcile with the reported large uncoupling between oxygen and glucose consumption (105) and explanations linking the lactate increase to brain glycogen (106) at present appear unlikely (see above). To address this question, it is useful to measure the TCA cycle activity in the brain. In intact tissue the transfer of ^{13}C label into the glutamate pool has been linked to TCA cycle flux. The rate of label incorporation indicated a significant difference in the rate of glutamate labeling with activation, but oxygen consumption increased at most by 30%, which is approximately half of the cerebral blood flow increase measured using this stimulation paradigm (107). This study supported the concept that oxygen consumption increases are less than the associated cerebral blood flow increases, leading to a net

decrease in deoxyhemoglobin content during focal activation, which forms the basis of blood-oxygen-level-dependent functional MRI (108).

Measurements of Krebs cycle flux from the flow of label from glucose C1 to glutamate C4 are affected by transport across the highly charged inner mitochondrial membrane. It is likely that such transport is controlled, since rapid transport of acids is unlikely and would interfere with chemiosmosis (109). Indeed, this exchange rate V_x was found to be comparable to the flux through pyruvate dehydrogenase, V_{PDH}, in most recent studies of the intact brain (48; 81; 110), consistent with what has been reported in most other tissues (76; 111-113). Many, but not all investigators have found that V_x is on the order of V_{PDH}, which implied that the malate-aspartate shuttle may be a major mechanism mediating the exchange of label across the mitochondrial membrane (30; 48; 81; 110; 111; 114-117).

Glutamategic neurotransmission and glial energy metabolism

Brain metabolism is exquisitely compartmentalized, with at least two major compartments (attributed to the "neuronal" and "glial" compartment) that are differentiated by the size of the respective glutamate pools associated with the Krebs cycle and that are metabolically linked by the so-called glutamate-glutamine cycle (118; 119). The scheme in Figure 10 summarizes some of the salient features that have been exploited by us and subsequently others in modeling brain metabolism.

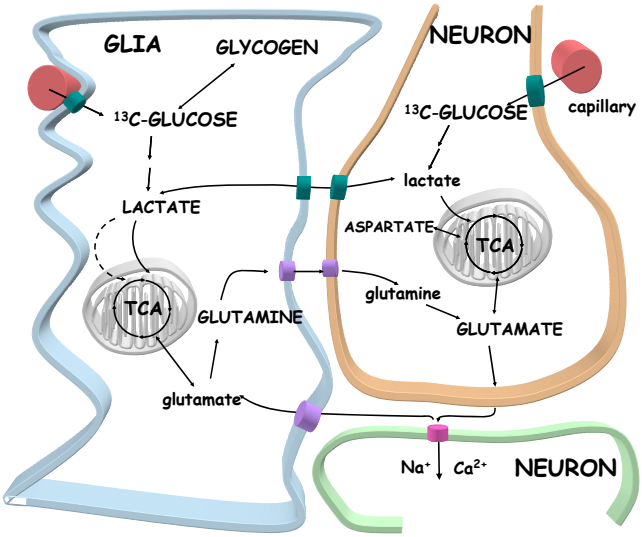

Figure 10. Compartmentation of cerebral neurotransmitter and amino acid metabolism studied via labeling from glucose at e.g. the C1 position. The glial compartment is on the left and the neuronal on the right. Anaplerosis, mediated by pyruvate carboxylase (dashed arrow), glutamine synthetase, as well as glycogen and its metabolism are localized to the glial compartment in adult brain. Most of glutamine is in the glial compartment and most of glutamate and aspartate in the neuronal compartment. The metabolites in caps give rise to NMR signals we have measured *in vivo*.

Key to cerebral compartmentation is the inactivation of neurotransmitter glutamate by uptake into perisynaptic astrocytes and conversion into electrophysiologically inactive glutamine which then diffuses back to the neuron (120-123). Clearly, this mechanism implies a much more active role for astrocytes than is conventionally assumed, since the conversion of neurotransmitter glutamate to glutamine invokes glial energy metabolism (124-126). The neuron-astrocyte pair thus has to be considered the functional unit (the "tripartite synapse" containing the pre- and postsynaptic neuron and the astrocyte) intimately involved in achieving chemical transmission as has been proposed in the last decade by Magistretti and others (127-129).

The link between astrocytes and neurons is generally accepted from a metabolic and a neurophysiological standpoint (130), yet differences exist as to the precise relationship between the metabolic rates and the specific energetics involved (48; 81; 117; 131; 132). The bulk of investigations suggest that non-signaling energy metabolism is approximately one-third to one half of that at resting (non-

stimulated) conditions (117; 133). Nonetheless, the controversial hypothesis put forth by Shulman et al. (131; 134) linking glucose metabolism with glutamatergic action is intriguing as it emphasizes further the coupling between neurons and glia at the level of energy metabolism as put forward earlier by others, e.g., (129).

Astrocytes have significant oxidative capacity to generate ATP, which can be substantiated with two experimental observations. First, anaplerosis, a mitochondrial reaction confined to the glial compartment, mediated by pyruvate carboxylase, is now generally accepted to have significant activity *in vivo* (11; 13; 48; 116; 117; 135; 136). To the extent that anaplerosis leads to net glutamine synthesis (via pyruvate carboxylase) in the glial compartment, a significant net synthesis of ATP occurs oxidatively, thereby generating approximately one third of the ATP per glucose molecule compared to that from complete glucose oxidation (116). Second, the presence of significant oxidative metabolism in astrocytes cn already be inferred from the fact that acetate is known to be exclusively metabolized in astrocytes by the TCA cycle (137; 138), which can only occur by oxidative metabolism (139).

OUTLOOK

As illustrated above and summarized in the abstract, NMR spectroscopy has already made many substantial contributions to our understanding of brain metabolism, e.g. understanding glucose transport, measuring brain glutamate and glutamine turnover, quantifying astrocytic and brain glycogen metabolism. Pivotal achievements include the demonstration that pyruvate carboxylase activity is substantial *in vivo*, and that glutamatergic neurotransmission can potentially be quantified *in vivo*. Future developments on the technical side probably will include the extension of these studies to GABA metabolism *in vivo*, and the characterization of the various fluxes that can be measured in different brain areas. Some investigators have already begun applying ^{13}C NMR spectroscopy to disease states in humans [88, 123] and animal models of disease,[154] and future expansion is to be expected in this area as well.

Deleted:

Field Code Changed

Deleted: ,